Atty. Docket No.: 204231/2055N PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Choong-Chin Liew	Examiner:	Juliet Switzer
Serial No.:	10/812,707		
Filed:	March 30, 2004	Group Art Unit:	1634
Titled:	Method for the Detection of Bladder Cancer Related Gene Transcripts in Blood		
		Conf. No.:	5409

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF Hongwei Zhang UNDER 37 C.F.R. §1.132

Sir:

I, **Hongwei Zhang**, Ph.D., hereby declare that:

1. I received a Ph.D. degree from the Institute of Medical Science at the University of Toronto in 2002, and a Master of Science degree from the Department of Immunology at the University of Toronto in 1995. In addition I received my Medical Degree from the University of Medical Sciences in Changchun China in 1989 and practiced as a staff physician for 4 years in Beijing prior to commencing my post graduate studies. I currently hold the position of Director of Biomarker Development at GeneNews Corporation (formerly ChondroGene Ltd., the Assignee of the application).

I am a trained molecular biologist experienced in developing methods to identify biomarkers which are indicative of a disease or condition, and in developing methods of using these biomarkers and products thereof as applied in the area of bladder cancer, amongst other conditions.

List of Publications:

K.W. Marshall, M.D., PhD., F.R.C.S., **H. Zhang, M.D., PhD.**, T.D. Yager Ph.D., N. Nossova M.D., Ph.D., A. Dempsey PhD., R. Zheng M.D., M. Han M.D. Ph.D., H.Tang M.Sc., S. Chao M.A.Sc, and C.C. Liew PhD. "Blood-based biomarkers for detecting mild osteoarthritis in the human knee" *OsteoArthritis and Cartilage (2005)* 861-871.

Zhang H, Marshall KW, Tang H, Hwang DM, Lee M, Liew CC. Profiling genes expressed in human fetal cartilage using 13,155 expressed sequence tags. Osteoarthritis Cartilage 2003;11:309-19.

Hongwei Zhang, C.C.Liew, K.Wayne Marshall. Microarray Analysis Reveals the Involvement of Beta-2 Microglobulin (B2M) in Human Osteoarthritis. Osteoarthritis and Cartilage 2002;10:950-60.

Doherty PJ, **Zhang H,** Manolopoulos V, Trogadis J, Tremblay L, Marshall KW. Adhesion of transplanted chondrocytes onto cartilage in vitro and in vivo. J Rheumatol 2000;27:1725-312.

Zhao YX, Lajoie G, **Zhang H,** Chiu B, Payne U, Inman RD. Tumor necrosis factor receptor p55-deficient mice respond to acute Yersinia enterocolitica infection with less apoptosis and more effective host resistance. Infect Immun 2000;68:1243-513.

Vaselios Manolopoulos, K. Wayne Marshall, **Hongwei Zhang,** Judy Trogadis, Louise Trembly and Paul J. Doherty. Factors affecting the efficacy of bovine chondrocyte transplantation in vitro. Osteoarthritis and Cartilage 1999;7:453-460.

Yi-Xue Zhao, **Hongwei Zhang,** Basil Chiu, Usulra Payne, Robert D. Inman. Tumor necrosis factor receptor P55 controls the severity of arthritis in experimental *Yersinia Enterocolitica* infection. Arthritis & Rheumatism 1999;42:1662-1672.

Paul J. Doherty, **Hongwei Zhang,** Louise Trembley, Vaselios Manolopoulos and K. Wayne Marshall. Resurfacing of articular cartilage explants with genetically-modified human chondrocytes *in vitro*. Osteoarthritis and Cartilage 1998;6:153-160.

Hongwei Zhang, Donna Phang, Ronald M. Laxer, Earl D. Silverman, Sueihua Pan, and Paul J. Doherty. Evolution of the T cell receptor beta repertoire from synovial fluid T cells of patient with juvenile onset rheumatoid arthritis. J. Rheumatol. 1997;24:1396-402.

Petro Lastres, Anihoa Letamendia, **Hongwei Zhang,** Carlos Rius, Nuria Almendro, UIIa RAab, Louis A. Lopez, Carmen Langa, Angels Fabra, Michelle Letarte and Carmelo Bernabeu. Endoglin modulates cellular responses to TGF-beta 1. J. Cell Biol. 1996;133:1109-1121.

Hongwei Zhang, Andrew R.E. Shaw, Allan Mak, and Michelle Letarte. Endoglin is a component of the Transforming Growth Factor (TGF)-beta receptor complex of human pre-B leukemic cells. J. Immunol. 1996,156:565-573.

2. I have read the non-final Office Action mailed May 11, 2007 in the above-referenced patent application.

In providing grounds for rejection of claims under 35 U.S.C. § 112(1), the Examiner asserts at page 8 of the Office Action: "*It is not known under what circumstances the result observed in the instantly examined control and test populations would be repeatable, as the results have not been validated.*"

3. As a scientist skilled in the area of molecular biomarker identification, I submit that post-filing validation experiments performed by the Assignee of the present application using both quantitative RT-PCR (QRT-PCR), an alternate technology relative to microarray analysis employed in the experiments disclosed at Example 19 of the specification, as well as an independent cohort of control and disease subjects relative to those employed in the experiments disclosed at Example 19 of the specification, have shown that RNA encoded by the gene TNFRSF7 is present at statistically lower levels in blood of subjects having bladder cancer relative to healthy control subjects.

Levels of TNFRSF7 expression in blood are statistically lower in bladder cancer patients versus healthy control subjects – validation of TNFRSF7 as biomarker of bladder cancer in blood via an alternate technology (quantitative RT-PCR) using an independent cohort

Attached as Exhibit "A" to this Declaration are results from post-filing experiments performed by the Assignee of the present application in which levels of TNFRSF7-encoded RNA in blood were found to be statistically lower in subjects having bladder cancer relative to healthy control subjects, as determined via an alternate technology relative to the microarray analysis employed in the experiments disclosed at Example 19 of the specification, i.e. via QRT-PCR analysis, and via an independent cohort of control and disease subjects relative to that described at Example 19 of the specification. As shown in Tables 1 and 2 of Exhibit "A", the average level of TNFRSF7-encoded RNA in blood samples from 22 bladder cancer patients, as determined via raw Ct values obtained from QRT-PCR analysis, was found to be 0.316-fold that of 18 healthy control subjects tested

(i.e. 3.2-fold lower), with the difference in expression levels being statistically significant (p = 0.041). Quantitative RT-PCR analysis was performed essentially as described in Osman *et al.*, of record.

In view of the above, I submit that the specification enables one of skill in the art to practice the claimed methods.

5. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that wilful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

Hongwei Zhang, Ph.D. _____

Date 2007-Nov-12

EXHIBIT "A"

TABLE 1. Quantitative RT-PCR analysis of TNFRSF7-encoded RNA levels in blood of bladder cancer patients vs healthy control subjects.

Experimental group	Sample ID	Relative level of TNFRSF7-encoded RNA (raw Ct)
Healthy control	HZ0091	24.95
	HZ0112	23.5
	IO057p	23.98
	IO069P	24.035
	IO070P	24.115
	IO071P	23.965
	IO072p	25.185
	IO073P	23.955
	N25C	22.995
	N74P	24.155
	N75P	11.82
	N77P	23.41
	N80AP	24.115
	N81AP	23.635
	PN0226	24
	PN0241	23.03
	PN0246	23.825
	PN0268	25.21
Bladder cancer	IO004P	26.735
	IO010P	25.885
	IO011P	27.555
	IO013P	27.95
	IO014P	24.89
	IO015P	24.85
	IO022P	26.275
	IO051P	23.95
	IO088P	24.34
	IO092P	24.075
	IO094P	25.02
	IO104BP	23.95
	PN0037	24.345
	PN0057	24.44
	PN0144	25.11
	PN0159	24.14
	PN0198	24.315
	PN0255	24.63
	PN0278	23.43
	PN0284	23.38
	PN0288	24.085
	PN0293	23.935

TABLE 2. Analysis of QRT-PCR data of Table 1, above, for bladder cancer detection.

Average level of TNFRSF7 expression in blood (raw Ct)	**healthy control subjects**	23.22
	bladder cancer patients	24.88
Average differential TNFRSF7 expression in blood (bladder cancer patients/control subjects)		0.316
p-value		0.041